

IN THE CLAIMS:

Please cancel claims 15-27 and 33.

Please amend claims 1, 9, 28 and 29 as follows:

1. [Amended] An aqueous composition buffered to a pH of from about 7 to about 9, and comprising, in the same solution:

a) first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides, and

b) third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands by from 90 to 400 nucleotides, each of said first, second, third and fourth primers having a  $T_m$  within the range of from about 65 to about  $74^{\circ}\text{C}$ , all of said primer  $T_m$ 's being within about  $50^{\circ}\text{C}$  of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides.

9. [Amended] A diagnostic test kit for the amplification of first and second target DNA's comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising, in the same solution:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides, and third and fourth

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cont.

primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides, each of said first, second, third and fourth primers having a  $T_m$  within the range of from about 65 to about 74°C, all of said primer  $T_m$ 's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

b) at least one additional PCR reagent.

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28. [Amended] The method of claim [15] 34 wherein three or more target DNA's are amplified using a set of primers for each of said target DNA's, the primers in each of said primer sets having a  $T_m$  within the range of [about] 65 to [about] 74°C, all of said primer  $T_m$ 's being within about 5°C of each other, and said primers in each primer set having nucleotide lengths with differ from each other by no more than 5 nucleotides.

29. [Amended] The method of claim 28 wherein each of said amplified target DNA's is captured with a capture reagent comprising a water-insoluble support to which is covalently attached a capture probe which is specific to a nucleic acid sequence of a strand of a distinct amplified target DNA strand, each capture probe having from 10 to 40 nucleotides and a  $T_m$  greater than about 50°C, and being hybridizable with said nucleic acid sequence of said distinct amplified target DNA strand at a temperature in the range of from [about] 40 to [about] 55°C.

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Please add new claims 34-36 as follows:

R1.126 -- 33. A method for the simultaneous amplification and detection of a first target DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from 7 to 9, and comprising, in the same solution:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides, third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides, each of said first, second, third and fourth primers having a  $T_m$  within the range of from 65 to 74°C, all of said primer  $T_m$ 's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being supplied in the same or a different composition as defined in i), to simultaneously amplify said opposing first target DNA

strands and said opposing second target DNA strands, provided that in each PCR cycle, each of priming and primer extension are carried out at a temperature within the range of from 62 to 75°C,

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B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.

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34 35. The method of claim 33 wherein three or more target DNA's are amplified using a set of primers for each of said target DNA's, the primers in each of said primer sets having a  $T_m$  within the range of from 65 to 74°C, all of said primer  $T_m$ 's being within about 5°C of each other, and said primers in each primer set having nucleotide lengths which differ from each other by no more than 5 nucleotides.

35 36. A method for the simultaneous amplification and detection of a first target DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from 7 to 9, and comprising, in the same solution:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides, third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated

from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides, each of said first, second, third and fourth primers having a  $T_m$  within the range of from 67 to 74°C, all of said primer  $T_m$ 's being within about 2°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being supplied in the same or a different composition as defined in i), to simultaneously amplify said opposing first target DNA strands and said opposing second target DNA strands, provided that in each PCR cycle, priming and primer extension are carried out at the same temperature within the range of from 62 to 75°C,

B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.

REMARKS

The foregoing amendments to the accompanying divisional application add a claim for priority to the earlier filed application, and cancel original claims 15-27 and 33, these claims having been allowed in parent application Serial No. 08/062,023, filed May 14, 1993.

Claims 1-14, 28-32 and 34-36 are now in this case. Claims 1, 9, 28 and 29 were amended as above in the amendment to the parent application Serial No. 08/062,023 dated November 5, 1993, then later canceled; claim 28 has been further amended hereinabove to correct claim dependency in light of change in claim numbering. Claim 34 is presented here as re-numbered